Antibody Blockade of Thrombospondin Accelerates Reendothelialization and Reduces Neointima Formation in Balloon-Injured Rat Carotid Artery

Donghui Chen, MD; Takayuki Asahara, MD; Kevin Krasinski, BA; Bernhard Witzenbichler, MD; Jihong Yang, MD; Meredith Magner, BA; Marianne Kearney, BS; William A. Frazier, PhD; Jeffrey M. Isner, MD; Vicente Andrés, PhD

Background—Remodeling of the extracellular matrix plays an important role during the pathogenesis of atherosclerosis and restenosis. The matrix glycoprotein thrombospondin-1 (TSP1) inhibits endothelial cell proliferation and migration in vitro. In contrast, TSP1 facilitates the growth and migration of cultured vascular smooth muscle cells. Accordingly, we investigated the hypothesis that administration of anti-TSP1 antibody could facilitate reendothelialization and inhibit neointimal thickening in balloon-injured rat carotid artery.

Methods and Results—Sprague-Dawley rats were subjected to left common carotid artery denudation, after which arteries were treated with C6.7 anti-TSP1 or control antibody. Evans blue dye staining 2 weeks after injury disclosed significantly increased reendothelialization in arteries treated with C6.7 antibody compared with the control group, and this effect was associated with increased number of proliferating cell nuclear antigen–positive endothelial cells. In contrast, treatment with C6.7 antibody decreased the number of proliferating cell nuclear antigen–positive vascular smooth muscle cells in the injured arterial wall. Neointimal thickening was correspondingly attenuated to a statistically significant degree in arteries receiving C6.7 antibody versus the control group at both the 2-week and 4-week time points.

Conclusions—Intra-arterial delivery of antibody against TSP1 facilitated reendothelialization and reduced neointimal lesion formation after balloon denudation. **(*Circulation*. 1999;100:849-854.)**

Key Words: antibodies ■ angioplasty ■ endothelium ■ carotid arteries ■ muscle, smooth

Endothelial dysfunction triggers a cascade of events that contribute to the pathogenesis of atherosclerosis and restenosis, including platelet activation and aggregation, vascular smooth muscle cell (VSMC) proliferation and migration, and deposition of extracellular matrix (ECM) components into the vessel wall.[1–6] It has become increasingly clear that multiple cytokines, in conjunction with the ECM and integrins, orchestrate vascular remodeling in response to arterial injury.[7–9]

See p 783

The vascular ECM is a complex of different macromolecules organized into a highly ordered architectural framework that provides the structural supporting element for the vascular cells and surrounding tissues. ECM components also participate in the regulation of other highly specialized cellular functions triggered by growth factors and cytokines, including cell adhesion, migration, proliferation, and differentiation.[7,10–12] The matrix protein thrombospondin-1 (TSP1) is synthesized and secreted by activated platelets[13] and a variety of cell types including endothelial cells (ECs),[14,15] macrophages,[16] fibroblasts,[17] and VSMCs.[18] TSP1 is a 450-kDa homotrimeric glycoprotein that interacts with multiple extracellular macromolecules and cell surface receptors, thus exerting a wide range of functions.[19,20] Cell culture experiments have demonstrated the ability of TSP1 to induce arrest of EC growth.[21,22] The spontaneous development of angiogenic tube-like structures is also inhibited by TSP1 both in vitro and in vivo.[23–25] In marked contrast, TSP1 promotes VSMC proliferation and migration[26,27] and plays a stimulatory role in platelet activation and aggregation.[28,29] These findings suggest that TSP1 may play an important role in the pathogenesis of atherosclerosis and restenosis. Consistent with this notion, TSP1 expression has been associated with atherosclerotic lesions, acute vascular injury, hypercholesterolemia, and hypertension.[15,30–35] In the present study, monoclonal neutralizing anti-TSP1 antibody was locally delivered to the arterial wall after balloon angioplasty in the rat carotid

Received January 11, 1999; revision received April 7, 1999; accepted April 22, 1999.
From the Department of Medicine (Cardiology), St Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Mass (D.C., T.A., K.K., B.W., J.Y., M.M., M.K., J.M.I., V.A.); the Unit of Vascular Biology, Instituto de Biomedicina, Consejo Superior de Investigaciones Científicas, Valencia, Spain (V.A.); and the Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, Mo (W.A.F.).
Correspondence to Vicente Andrés, PhD, Instituto de Biomedicina (C.S.I.C.), Jaime Roig 11, 46010 Valencia, Spain. E-mail vandres@ibv.csic.es

Circulation **is available at http://www.circulationaha.org**

artery to evaluate in vivo the role of TSP1 on vascular repair after acute injury. We show that local administration of anti-TSP1 antibody expedited reendothelialization and reduced neointimal thickening.

Methods

Balloon Angioplasty, Antibody Delivery, and Evaluation of Reendothelialization and Neointimal Thickening

Balloon angioplasty in the left common carotid artery of male Sprague-Dawley rats was performed essentially as described by Clowes et al.[2] Immediately after angioplasty, protein A–purified mouse monoclonal anti-TSP1 antibody C6.7[29] or control nonspecific IgG antibody MOPC-21 (Sigma Chemical) was delivered intra-arterially by use of the dwell technique (100 μg of IgG in 100 μL of sterile 0.1% BSA/PBS; 30 minutes), This was followed by 1 week of continuous periarterial delivery by osmotic pump (Alzet, model 2 ML1, Alza) as described previously.[36,37] In brief, the common carotid artery was further isolated and dissected free of fat and connective tissue and a superficial longitudinal incision into the adventitia was made with a modified coronary artery surgical blade to ensure penetration of the antibody to the external muscular layer of the media. A sterile microcatheter was placed adjacent to the injured portion of the artery and secured by suturing it directly to the adjacent musculature. The proximal end of the microcatheter was heat-sealed, and longitudinal perforations were made on the catheter at the site adjacent to the injured arterial segment. The distal end of the microcatheter was passed through the lateral neck and connected to the osmotic pump. The pump was filled with C6.7 or MOPC-21 control antibody (400 μg IgG in a final volume of 2 mL of sterile PBS). The wounds were cleaned with saline and wiped dry with a sterile cotton swab. Fascia surrounding the artery was sutured closed. The pump was placed and sutured in a pocket made in the back of the rat. One week after infusion at 10 μL/h, the pump and the microcatheter were removed from the rats under anesthesia.

The study comprised 13 arteries treated with C6.7 antibody (n=7, 2 weeks; n=6, 4 weeks) and 18 arteries treated with control antibody (n=9, 2 weeks; n=9, 4 weeks). Two carotid arteries from the control group (1 at each time point) developed thrombosis after balloon injury and were therefore discarded. All vessels treated with C6.7 antibody were patent at the time the animals were killed. Animals received an intravenous injection of 1 mL 0.5% Evans blue 30 minutes before they were killed to identify nonendothelialized surfaces. The extent of reendothelialization and neointimal thickening 2 and 4 weeks after angioplasty was evaluated by planimetric analysis as previously described.[38]

To assess antibody delivery into the arterial wall, animals were killed 3 days after angioplasty. Methanol-fixed arteries were sectioned longitudinally for immunohistochemistry with the use of rat-adsorbed, biotinylated horse anti-mouse antibody (Vector Laboratories) and a biotin/streptavidin-horseradish peroxidase detection system (Signet Laboratories). A polyclonal horse anti-goat antibody was used for negative control.

Antibody Bioassay

VSMC migration was assessed with a modified Boyden chamber (Neuroprobe) and platelet-derived growth factor (PDGF) BB (10 ng/mL) as the chemoattractant.[39] Rat aortic VSMCs were isolated as previously described,[40] seeded in the upper compartment (2.5×10^5 cells in 50 μL of 1% FBS/DMEM), and incubated for 30 minutes in the absence or in the presence of C6.7 antibody. After 5 hours of incubation, migration was quantified by counting the number of cells on the lower side of the filter from 3 randomly chosen high-power ($\times$400) fields.

Expression Studies and Assessment of Proliferative Activity After Balloon Injury

Arteries were harvested and adventitia and connective tissues were removed as cleanly as possible. Preparation of arterial extracts and Western blot analysis were carried out as previously described.[41] Blots were probed with rabbit polyclonal anti-TSP1 antibody (a gift from Dr Jack Lawler) and mouse monoclonal anti-tubulin antibody (Calbiochem). After incubation with horseradish-peroxidase–conjugated secondary antibodies, immunocomplexes were visualized with ECL reagent (Amersham). Proliferative activity in injured arteries was evaluated by immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) expression as previously described.[38] ECs were identified by immunohistochemistry with the use of a mouse monoclonal anti-CD31 antibody (1:40 dilution in 1% BSA/PBS) (PharMingen).

Statistical Analysis

Results are expressed as mean±SEM. Differences in the number of migrating cells and PCNA-immunoreactive cells were evaluated by use of a 2-tailed, unpaired Student's *t* test. Differences in the extent of reendothelialization and neointimal thickening were evaluated by use of ANOVA and Scheffé's *F* post hoc test. Differences were considered significant at $P<0.05$.

Results

Induction of TSP1 Expression After Balloon Angioplasty and Biological Activity of C6.7 Antibody

TSP1 protein expression in the rat carotid artery was greatly upregulated as early as 1 day after angioplasty, and its expression was maintained up to 2 weeks (Figure 1A). These findings are consistent with previous Northern blot and immunohistochemical analysis demonstrating a rapid induction of TSP1 in balloon-injured arteries.[33,34,42]

We next evaluated the biological activity of neutralizing anti-TSP1 antibody C6.7. Treatment with C6.7 antibody inhibited in a dose-dependent manner PDGF BB–dependent migration of cultured VSMCs (Figure 1B). When C6.7 antibody solution containing 50 μg/mL IgG was incubated for 6 days at 37°C, its inhibitory effect on PDGF BB–mediated VSMC migration was comparable to that elicited by the same concentration of fresh antibody. Thus prolonged incubation of C6.7 antibody at body temperature did not appear to reduce its biological activity.

C6.7 Antibody Delivery Facilitates Reendothelialization After Balloon Angioplasty

To assess the role of TSP1 on the vascular response to injury, neutralizing C6.7 antibody was delivered intra-arterially by use of the dwell technique followed by continuous delivery with an osmotic pump. We first evaluated the presence of mouse monoclonal C6.7 antibody in the injured arterial wall 3 days after angioplasty. Longitudinal sections were incubated with horse anti-mouse IgG, which elicited immunoreactivity within the media and adventitia (Figure 1C, left). No signal was detected when horse anti-goat IgG was used as the secondary antibody (Figure 1C, right). These studies demonstrated the presence of mouse monoclonal C6.7 antibody in the injured arterial wall.

To evaluate the effect of C6.7 antibody on reendothelialization and neointimal thickening, rats were implanted with the osmotic pump delivery system for 1 week after balloon angioplasty. Animals received an intravenous injection of 1 mL 0.5% Evans blue dye before they were killed to evaluate the extent of reendothelialization (Figure 2A). Two weeks after angioplasty, C6.7-treated arteries disclosed a 60% in-

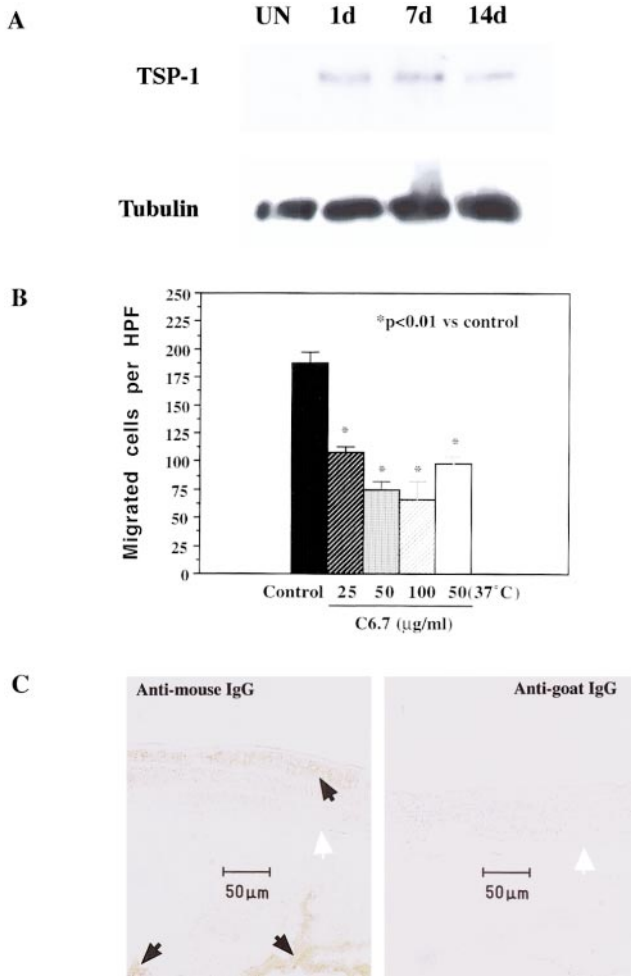

Figure 1. TSP1 expression and C6.7 antibody delivery after balloon injury. A, Tissue extracts were prepared from uninjured (UN) arteries and at the indicated times after angioplasty. TSP1 and tubulin expression was evaluated by Western blot analysis. B, Rat VSMC migration was evaluated with the use of a Boiden chamber and PDGF BB as chemoattractant. Cells were treated with the indicated concentrations of fresh C6.7 antibody or with C6.7 antibody that had been previously incubated at 37°C for 6 days (open bar). After 5 hours of incubation, cells were counted in 3 randomly chosen high power fields (HPF) from the lower side of each filter. All assays were performed in triplicate. C, Arterial sections were incubated with horse anti-mouse secondary antibody to assess the presence of mouse monoclonal C6.7 antibody (black arrows). No signal was detected when using horse anti-goat secondary antibody. White arrows point to the external elastic lamina.

crease in reendothelialized area as compared with the control group (46.17±2.04% in the control group vs 73.31±3.26 in the C6.7 group, $P=0.0016$). No statistically significant differences between the control group and the C6.7 group were seen at 4 weeks after angioplasty.

Effect of C6.7 Antibody Delivery on Cellular Proliferation and Neointimal Thickening

We have previously shown that PCNA expression after balloon angioplasty correlates temporally and spatially with cellular proliferation in the rat carotid artery.[43] Thus PCNA immunostaining was used to assess the effect of C6.7 antibody on cellular proliferation in balloon-injured arteries. Two weeks after angioplasty, C6.7-treated arteries disclosed a higher number of PCNA-positive cells at the luminal surface (1.57±0.26 mm^{-1} in the control group vs 3.09±0.49 mm^{-1} in the C6.7 group, $P<0.05$) (Figure 2, C and D). Adjacent sections were analyzed with anti-CD31 antibody to identify ECs. In agreement with the results of Evans blue staining, CD31 immunoreactivity at the luminal edge of the neointima was more abundant in arteries treated with C6.7 antibody than in control arteries (Figure 2D, and data not shown). Thus accelerated reendothelialization after balloon angioplasty on administration of C6.7 antibody appeared to correlate with increased EC proliferation.

Consistent with the kinetics of proliferation in balloon-injured rat carotid arteries,[43,44] PCNA immunolocalization at 2 weeks after angioplasty was limited predominantly to the neointima (Figure 2D). Treatment with C6.7 antibody reduced the number of neointimal PCNA-positive VSMCs by approximately half (23.6±4.08 mm^{-1} in the control group vs 11.92±2.46 mm^{-1} in the C6.7 group, $P<0.05$) (Figure 2C). As expected, the number of PCNA-positive cells at 4 weeks was reduced in the neointima of both control and C6.7-treated arteries (data not shown).

Collectively, the above results suggest that administration of anti-TSP1 antibody inhibited VSMC hyperplasia in vivo. The intima-to-media (I/M) ratio was correspondingly reduced by ≈63% in the C6.7 group at both the 2- and 4-week time points (1.33±0.04 in the control group vs 0.83±0.05 in the C6.7 group at 2 weeks, $P<0.0001$; 1.57±0.07 in the control group vs 0.94±0.04 in the C6.7 group at 4 weeks, $P<0.0001$) (Figure 2B). Of note, although control arteries showed a statistically significant higher I/M ratio at 4 weeks as compared with the 2-week time point ($P=0.03$), neointimal thickening in the C6.7-treated arteries remained nearly unchanged during the same time interval.

Discussion

The expression pattern of TSP1 is consistent for a role of this matrix protein on the pathogenesis of atherosclerosis and restenosis[15,30–35] (this study). Previous in vitro studies also support this notion. For example, TSP1 inhibits migration and proliferation of cultured ECs,[21,22] and several studies have suggested that this might translate into inhibition of angiogenesis in vivo.[23,24,45–47] The extent to which these observations may be extrapolated to reendothelialization after balloon injury, however, has never been tested. Given the ability of TSP1 to promote VSMC proliferation and migration in vitro,[26,27] TSP1 would potentially represent a unique matrix protein with dual effects on ECs and VSMCs that might both delay EC regeneration and facilitate neointimal lesion formation after balloon injury. The present study demonstrates that administration of the anti-TSP1 antibody C6.7 after balloon angioplasty in the rat carotid artery expedited reendothelialization and reduced neointimal thickening, and these effects were associated with increased EC proliferation and reduced VSMC growth.

Recent studies have demonstrated direct effects of TSP1 on ECs that are consistent with our observations. For example, addition of TSP1 to ECs that had already formed

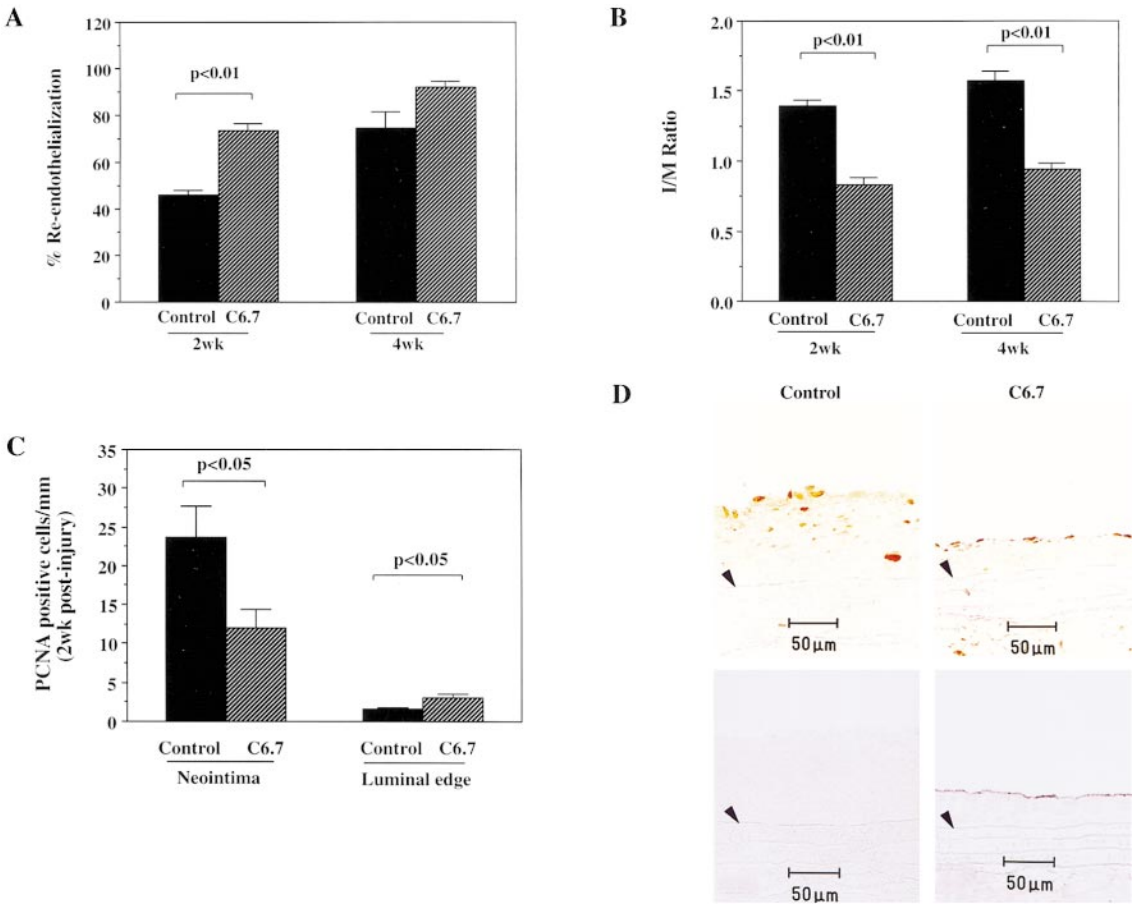

Figure 2. Effect of C6.7 antibody on reendothelialization, neointimal thickening, and cellular proliferation in balloon-injured rat carotid arteries. Computer-assisted quantitative morphometry performed by a blinded observer disclosed statistically significant accelerated reendothelialization (A) and reduced I/M ratio (B) in the C6.7 group 2 weeks after angioplasty. I/M ratio was also reduced to a statistically significant degree in the C6.7 group vs the control group at 4 weeks. Although control arteries showed a statistically significant higher I/M ratio at 4 weeks as compared with the 2-week time point, neointimal thickening in the C6.7-treated arteries remained nearly unchanged at 4 weeks. C, PCNA-immunoreactive cells at 2 weeks after angioplasty. D, Representative microphotographs in control and C6.7 groups 2 weeks after injury. Adjacent sections to those used for PCNA immunostaining were incubated with anti-CD31 antibodies. Note abundant CD31 expression at the luminal edge of C6.7-treated arteries as compared with the control group, indicating higher number of ECs. Therefore, the number of PCNA-positive cells was scored within the neointima (predominantly VSMCs) and at the luminal edge (predominantly ECs). Black arrows point to the internal elastic lamina. C6.7 group: n=7 (2 weeks) and n=6 (4 weeks); control group: n=8 (2 weeks) and n=8 (4 weeks).

stable focal adhesions on a fibronectin substrate stimulated focal adhesion disassembly.[48] Moreover, TSP1 inhibited angiogenesis both in vitro and in vivo,[23–25] and some observations suggested that TSP1 might act as a physiological inhibitor of angiogenesis.[24,45–47] The ability of C6.7 antibody to inhibit neointimal thickening after balloon angioplasty is also in agreement with previous in vitro studies demonstrating a direct role of TSP1 in promoting VSMC growth and migration.[26,27,49–51]

In addition to the direct effect of TSP1 on VSMC proliferation, it is noteworthy to point out that several studies in the rat carotid model of vascular injury have clearly established an inverse relation between endothelial integrity and VSMC proliferation.[52–55] Moreover, application of various EC mitogens that accelerated reendothelialization attenuated neointimal lesion formation.[38,56,57] Thus expeditious reendothelialization in C6.7-treated arteries probably contributed to reduced neointimal thickening.

In conclusion, this study demonstrates a favorable effect of anti-TSP1 antibody on both reendothelialization and neointimal hyperplasia after balloon angioplasty. Thus the results imply the potential utility of a novel treatment strategy in which inhibition of a matrix protein simultaneously promotes EC growth and reendothelialization and inhibits VSMC proliferation. This could be a powerful therapeutic strategy to inhibit neointimal thickening after balloon denudation.

Acknowledgments

This study was supported by National Institutes of Health grants HL-57519 and AG-15227 (V.A.); HL-40518, HL-57516, and HL-53354 (J.M.I.); and CA-65872 (W.A.F.). We are grateful to Mickey Neely for assistance in preparing the manuscript and to Jack Lawler for the gift of polyclonal anti-TSP1 antibodies.

References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature.* 1993;362:801–809.

2. Clowes AW, Reidy MA, Clowes MM. Mechanisms of stenosis after arterial injury. *Lab Invest*. 1983;49:208–215.
3. Gibbons GH, Dzau VJ. Molecular therapies for vascular diseases. *Science*. 1996;272:689–693.
4. Nikkari ST, Jarvelainen HT, Wight TN, Ferguson M, Clowes AW. Smooth muscle cell expression of extracellular matrix genes after arterial injury. *Am J Pathol*. 1994;144:1348–1356.
5. Strauss BH, Chisholm RJ, Keeley FW, Gotlieb AI, Logan RA, Armstrong PW. Extracellular matrix remodeling after balloon angioplasty injury in a rabbit model of restenosis. *Circ Res*. 1994;75:650–658.
6. Andrés V. Control of vascular smooth muscle cell growth and its implication in atherosclerosis and restenosis. *Int J Mol Med*. 1998;2:81–89.
7. Carey DJ. Control of growth and differentiation of vascular cells by extracellular matrix proteins. *Ann Rev Physiol*. 1991;53:161–177.
8. Wight TN. The extracellular matrix and atherosclerosis. *Curr Opin Lipidol*. 1995;6:326–334.
9. Assoian RK, Marcantonio EE. The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis. *J Clin Invest*. 1996;98:2436–2439.
10. Juliano RL, Haskill S. Signal transduction from the extracellular matrix. *J Cell Biol*. 1993;120:577–585.
11. Savani RC, Wang C, Yang B, Zhang S, Kinsella MG, Wight TN, Stern R, Nance DW, Turley EA. Migration of bovine aortic smooth muscle cells after wounding injury: the role of hyaluronan and RHAMM. *J Clin Invest*. 1995;95:1158–1168.
12. Ruoslathi E, Yamaguchi Y. Proteoglycans as modulators of growth factor activities. *Cell*. 1991;64:867–869.
13. Lawler J, Slayter HS, Coligan JE. Isolation and characterization of a high molecular weight glycoprotein from human blood platelets. *J Biol Chem*. 1978;253:8609–8616.
14. McPherson J, Sage H, Bornstein P. Isolation and characterization of a glycoprotein secreted by aortic endothelial cells in culture: apparent identity with platelet thrombospondin. *J Biol Chem*. 1981;256:11330–11336.
15. Reed MJ, Iruela-Arispe L, O'Brien ER, Truong T, LaBell T, Bornstein P, Sage EH. Expression of thrombospondins by endothelial cells: injury is correlated with TSP-1. *Am J Pathol*. 1995;147:1068–1080.
16. Jaffe EA, Ruggiero JT, Falcone DJ. Monocytes and macrophages synthesize and secrete thrombospondin. *Blood*. 1985;65:79–84.
17. Jaffe EA, Ruggiero JT, Leung LK, Doyle MJ, McKeown-Longo PJ, Mosher DF. Cultured human fibroblasts synthesize and secrete thrombospondin and incorporate it into extracellular matrix. *Proc Natl Acad Sci U S A*. 1983;80:998–1002.
18. Mumby SM, Abbott Brown D, Raugi D, Bornstein P. Regulation of thrombospondin secretion by cells in culture. *J Cell Physiol*. 1984;120:280–288.
19. Frazier WA. Thrombospondins. *Curr Opin Cell Biol*. 1991;3:792–799.
20. Asch AS, Tepler J, Silbiger S, Nachman RL. Cellular attachment to thrombospondin: cooperative interactions between receptor systems. *J Biol Chem*. 1991;266:1740–1745.
21. Bagavandoss P, Wilks JW. Specific inhibition of endothelial cell proliferation by thrombospondin. *Biochem Biophys Res Commun*. 1990;170:867–872.
22. Taraboletti G, Roberts D, Liotta LA, Giavazzi R. Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: a potential angiogenesis regulatory factor. *J Cell Biol*. 1990;111:765–772.
23. Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, Frazier WA, Bouck NP. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci U S A*. 1990;87:6624–6628.
24. Iruela-Arispe ML, Bornstein P, Sage H. Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. *Proc Natl Acad Sci U S A*. 1991;88:5026–5030.
25. O'Shea KS, Dixit VM. Unique distribution of extracellular matrix component thrombospondin in the developing mouse embryo. *J Cell Biol*. 1988;101:2737–2748.
26. Majack RA, Cook SC, Bornstein P. Control of smooth muscle cell growth by components of the extracellular matrix: autocrine role for thrombospondin. *Proc Natl Acad Sci U S A*. 1986;83:9050–9054.
27. Yabkowitz RY, Mansfield PJ, Ryan US, Suchard SJ. Thrombospondin mediates migration and potentiates platelet-derived growth factor-dependent migration of calf pulmonary artery smooth muscle cells. *J Cell Physiol*. 1993;157:24–32.
28. Tuszynski GP, Rothman LV, Murphy A, Siegler K, Knudsen KA. Thrombospondin promotes platelet aggregation. *Blood*. 1988;72:109–115.
29. Dixit VM, Haverstick DM, O'Rourke KM, Hennessy SW, Grant GA, Santoro SA, Frazier WA. Monoclonal antibodies against human thrombospondin inhibit platelet aggregation. *Proc Natl Acad Sci U S A*. 1985;82:3472–3476.
30. Wight TN, Raugi GJ, Mumby SM, Bornstein P. Light microscopic immunolocation of thrombospondin in human tissues. *J Histochem Cytochem*. 1985;33:295–302.
31. Liau G, Winkles JA, Cannon MS, Kuo L, Chilian WM. Dietary-induced atherosclerotic lesions have increased levels of acidic FGF mRNA and altered cytoskeletal and extracellular matrix mRNA expression. *J Vasc Res*. 1993;30:327–332.
32. Van Zanten GH, de Graaf S, Slootweg PJ, Heijnen HFG, Connolly TM, de Groot PG, Sixma JJ. Increased platelet deposition on atherosclerotic coronary arteries. *J Clin Invest*. 1994;93:615–632.
33. Roth JJ, Gahtan V, Brown JL, Gerhard C, Swami VK, Rothman VL, Tulenko TN, Tuszynski GP. Thrombospondin-1 is elevated with both intimal hyperplasia and hypercholesterolemia. *J Surg Res*. 1998;74:11–16.
34. Raugi GJ, Mullen JS, Barb DH, Okada T, Mayberg MR. Thrombospondin deposition in rat carotid artery injury. *Am J Pathol*. 1990;137:179–185.
35. Botney MD, Kaiser LR, Cooper JD, Mecham RP, Parghi D, Roby J, Parks WC. Extracellular matrix protein gene expression in atherosclerotic hypertensive pulmonary arteries. *Am J Pathol*. 1992;140:357–364.
36. Cuevas D, Gonzalez AM, Carceller F, Baird A. Vascular response to basic fibroblast growth factor when infused onto the normal adventitia or into the injured media of the rat carotid artery. *Circ Res*. 1991;69:360–369.
37. Tsurumi Y, Murohara T, Krasinski K, Chen D, Witzenbichler B, Kearney M, Couffinhal T, Isner JM. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. *Nat Med*. 1997;3:879–886.
38. Asahara T, Bauters C, Pastore C, Kearney M, Rossow S, Bunting S, Ferrara N, Symes JF, Isner JM. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. *Circulation*. 1995;91:2793–2801.
39. Witzenbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J Biol Chem*. 1998;273:18514–18521.
40. Pickering JG, Weir L, Rosenfield K, Stetz J, Jekanowski J, Isner JM. Smooth muscle cell outgrowth from human atherosclerotic plaque: implications for the assessment of lesion biology. *J Am Coll Cardiol*. 1992;20:1430–1439.
41. Chen D, Krasinski K, Chen D, Sylvester A, Chen J, Nisen PD, Andrés V. Downregulation of cyclin-dependent kinase 2 activity and cyclin A promoter activity in vascular smooth muscle cells by $p27^{KIP1}$, an inhibitor of neointima formation in the rat carotid artery. *J Clin Invest*. 1997;99:2334–2341.
42. Miano JM, Vlasic N, Tota RR, Stemerman MB. Smooth muscle cell immediate-early gene and growth factor activation follows vascular injury: a putative in vivo mechanism for autocrine growth. *Arterioscler Thromb*. 1993;13:211–219.
43. Wei GL, Krasinski K, Kearney M, Isner JM, Walsh K, Andrés V. Temporally and spatially coordinated expression of cell cycle regulatory factors after angioplasty. *Circ Res*. 1997;80:418–426.
44. Clowes AW, Clowes MM. Kinetics of cellular proliferation after arterial injury, II: inhibition of smooth muscle growth by heparin. *Lab Invest*. 1985;52:611–616.
45. Weinstat-Saslow DL, Zabrenetzky VS, Van Houtte K, Frazier WA, Robert DD, Steeg PS. Transfection of thrombospondin 1 cDNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res*. 1994;54:6504–6511.
46. RayChaudhury A, Frazier WA, D'Amore PA. Comparison of normal and tumorigenic cells: differences in thrombospondin production and

responses to transforming growth factor-beta. *J Cell Sci*. 1994;107: 39–46.
47. Canfield AE, Schor AM, Schor SL, Grant ME. The biosynthesis of extracellular-matrix components by bovine retinal endothelial cells displaying distinctive morphological phenotypes. *Biochem J*. 1986; 235:375–383.
48. Murphy-Ullrich JE, Hook M. Thrombospondin modulates focal adhesions in endothelial cells. *J Cell Biol*. 1989;109:1309–1319.
49. Majack RA, Goodman LV, Dixit VM. Cell surface thrombospondin is functionally essential for vascular smooth muscle cell proliferation. *J Cell Biol*. 1988;106:415–422.
50. Majack RA, Mildbrandt J, Dixit VM. Induction of thrombospondin messenger RNA levels occurs as an immediate primary response to platelet-derived growth factor. *J Biol Chem*. 1987;262:8821–8825.
51. Majack RA, Cook SC, Bornstein P. Platelet-derived growth factor and heparin-like glycosaminoglycans regulate thrombospondin synthesis and deposition in the matrix by smooth muscle cells. *J Cell Biol*. 1985;101:1059–1071.
52. Haudenschild CC, Schwartz SM. Endothelial regeneration, II: restitution of endothelial continuity. *Lab Invest*. 1979;41:407–418.
53. Clowes AW, Collazzo RE, Karnovsky MJ. A morphologic and permeability study of luminal smooth muscle cells after arterial injury in the rat. *Lab Invest*. 1978;39:141–150.
54. Fishman JA, Ryan GB, Karnovsky MJ. Endothelial regeneration in the rat carotid artery and the significance of endothelial denudation in the pathogenesis of myointimal thickening. *Lab Invest*. 1975;32:339–351.
55. Bjorkerud S, Bondjers G. Arterial repair and atherosclerosis after mechanical injury, V: tissue response after induction of a large superficial transverse injury. *Atherosclerosis*. 1973;18:235–255.
56. Bjornsson TD, Dryjski M, Tluczek J, Mennie R, Ronan J, Mellin TN, Thomas KA. Acidic fibroblast growth factor promotes vascular repair. *Proc Natl Acad Sci U S A*. 1991;88:8651–8655.
57. Meurice T, Bauters C, Auffray JL, Vallet B, Hamon M, Valero F, Von Belle E, Lablanche JM, Bertrand ME. Basic fibroblast growth factor restores endothelium-dependent responses after balloon injury of rabbit arteries. *Circulation*. 1996;93:18–22.